An Interaction Between Dietary Silicon and Arginine Affects Immune Function Indicated by Con-A-Induced DNA Synthesis of Rat Splenic T-Lymphocytes[†]

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ABSTRACT

Sporadic reports have appeared that suggest silicon plays a functional role in immune function by affecting lymphocyte proliferation. In addition, there is also considerable interest in supplemental arginine as a modulator of immune function. Therefore, the purpose of this animal experiment was to determine the effect of supplemental compared to adequate arginine on immune function as measured by splenic T-lymphocyte proliferation in the presence of adequate or inadequate dietary silicon. The independent variables were, per gram of fresh diet, silicon supplements of 0 or 35 μg and arginine supplements of 0 or 5 mg. The basal diet contained 2.3 μg silicon/g and 7.82 mg L-arginine/g. After feeding the male rats (nine per treatment group) for 8 wk, spleen lymphoid cells were isolated and cultured with methyl-^{3}[H]thymidine. Supplemental arginine significantly decreased Con-A-induced DNA synthesis of splenic T-lymphocytes, but the response to arginine was influenced by dietary silicon. The decreased DNA synthesis

was more marked when rats were fed adequate silicon than when fed inadequate silicon. Also, when arginine was not supplemented, DNA synthesis was higher in lymphocytes from rats fed an adequate silicon diet than rats fed the inadequate silicon diet. These findings support the hypothesis that an interaction between silicon and arginine affects immune function and that inadequate dietary silicon impairs splenic lymphocyte proliferation in response to an immune challenge.

Index Entries: Silicon; T-lymphocyte proliferation; immunity; trace elements; arginine.

INTRODUCTION

The scientific literature abounds with reports showing that silica (SiO_2) affects various cellular immune responses, especially inhaled silica, which leads to lung injury *(1).* Investigations of these immune responses have primarily focused on the generation of free radicals and nuclear factor-kappa B (NF-κ B) with silica-induced tumor necrosis factor-α (TNF-α) gene expression leading to fibrosis *(2).* Typically, the investigations of silicon in physiological concentrations on cellular immune function have involved lymphocyte cell culture; these indicate that silicon activates lymphocyte proliferation, whereas silicon inadequacy inhibits it. Henrotte et al. *(3)* cultured human lymphocytes and LDV/7 lymphoblast cells in the presence of monomethylsilanetriol (silanol), a soluble organic form of silicon and serine. Silanol stimulated peripheral lymphocyte proliferation but decreased lymphoblast proliferation at an optimal concentration of 10 mg of silicon per liter of culture media. Based on the increased proliferation finding, Henrotte et al. *(3)* suggested that silicon had a regulatory role in the cell cycle of lymphocytes. Yamaguchi et al. *(4)* utilized ^{45}Ca to determine that in vitro silica apparently spurred the immune system by activating human lymphocytes. One to five minutes after incubation with silica, intracellular Ca^{2+} increased markedly, which signaled activation of lymphocytes in vitro. The proliferation of lymphocytes, apparently mediated by silicon, has led researchers to conjecture about the functional role of silicon in normal cellular immunity.

In humans, arginine supplementation has been reported to enhance the T-lymphocyte response to mitogens; this indicates that arginine has the potential to affect the immune response. Our interest in investigating the possibility that silicon and arginine interact to affect the immune response was our preliminary finding that plasma silicon concentrations were decreased by arginine supplementation. Thus, the experiment described in this article was designed to determine the effects of supplemental arginine compared to adequate dietary arginine on immune function as measured by T-lymphocyte response in the presence of adequate or inadequate dietary silicon; that is, whether the intake of additional arginine would affect the immune response of rats subjected to silicon deprivation.

MATERIALS AND METHODS

Thirty-six Sprague-Dawley (Sasco, Omaha, NE) weanling male rats (aged 21 d) were weighed upon arrival and housed three per all-plastic cage measuring $50 \times 24 \times 16$ cm *(5)* and located inside a laminar airflow rack (Lab Products, Maywood, NJ). Rats were randomly assigned to treatment groups with no significant differences in weight (mean of 39 g) in a 2×2 factorial arrangement. The independent variables, or factors, were per gram of fresh diet, silicon as reagent-grade sodium metasilicate (J. T. Baker, Phillipsburg, NJ) supplements of 0 (–Si) or 35 μg (+Si), and arginine supplements (Ajinomoto U.S.A., Inc, Teaneck, NJ) of 0 and 5 mg. Because the basal diet contained 7.82 mg arginine/g, and the arginine requirement of the growing rat is 4.3 mg/g *(6)*, the supplemented rats were fed about three times the requirement.

The composition of the basal diet has been reported *(7)*. Analysis indicated that the basal diet contained 2.3 μg Si/g. Fresh food in plastic cups was provided *ad libitum* each day. The diets were mixed 1 wk before the start of the experiment. The diets were not pelleted and were stored at –16°C in tightly capped plastic containers. The rats were provided deionized water (Super Q System; Millipore Corp., Bedford, MA) in plastic cups. Absorbent paper under the false-bottom cages was changed daily. Room temperature was maintained at 23°C. Room lighting was controlled automatically to provide 12 h each of light and dark. Animals were weighed and provided clean cages weekly.

The rats were fed their respective diets for 8 wk. Following a 16-h fast, animals were weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination with a heparin-coated syringe and needle. The spleen was removed from each animal by aseptic techniques and was immediately processed. The liver was removed from each rat and frozen until processing for mineral analysis.

Spleen lymphoid cells (SLCs) were isolated by using a method previously described *(8)* but with minor modifications. Spleens were freed of extraneous tissue, then teased with sterile stainless-steel needles in Hanks balanced salt solution (HBSS) containing gentamicin sulfate (Gibco, Grand Island, NY). SLCs were separated from other spleen cells by density gradient centrifugation according to the Ficoll sodium metriozate method of Boyum *(9)*. Ten milliliters of the spleen cell suspension in HBSS were placed on 3 mL of Ficoll-Hypaque (Histopaque 1077; Sigma Chemical Co., St. Louis, MO) and centrifuged at room temperature at 400 *g* for 30 min. The isolated SLC suspensions were washed with 10 mL of HBSS three times and centrifuged after each wash. Viable cells (trypan blue exclusion) were counted by a hemocytometer.

Spleen lymphoid cells were cultured in RPMI-1640 tissue culture medium containing 2% heat-inactivated fetal bovine serum. The culture medium also contained L-glutamine (2.0 mmol/L) and gentamicin sulfate (12.5 mg/mL). In a total volume of 200 μL/well, 2×10^5 SLCs were incu-

bated in a flat-bottom Falcon Micro-Test plate (Micro-Test III; Falcon Plastics, Oxnard, CA) for 72 h at 37°C in a 5% CO_2, 95% humidified air incubator.

Triplicate control (without mitogen) cultures were evaluated for each SLC sample; triplicate test (with mitogen) cultures were also used for each mitogen dose tested. Con-A (concanavalin-A; Sigma Chemical Co., St. Louis, MO) was added to the test cultures at 0.1, 0.2, 0.39, and 0.78 μg/culture. After 48 h of incubation, 1.0 μCi of methyl-^{3}[H]thymidine (DuPont Co., Boston MA) was added to each control and test culture. Following 24 h of isotope labeling, the cultures were removed from the incubator, frozen, and then harvested with a Titertek Cell Harvester (Flow Laboratories, Inc., Rockville MD). The cell culture fluid, cells, and distilled water rinses were collected on glass-fiber harvester filters. Distintegrations per minute (DPM) in the harvested cells were determined by standard liquid scintillation counting procedures using Ready Protein Scintillation Fluid and a Beckman LS 6800 scintillation counter (Beckman, Fullerton, CA).

The air-dried basal diets and vacuum-dried liver samples were ashed in platinum crucibles at 450°C by a lithium–boron fusion technique *(10)*. Liver and dietary macrominerals and trace elements were determined by inductively coupled argon plasma–atomic emission *(11)*. Standard reference materials (National Institute of Standards and Technology, Gaithersburg, MD) #1572 Citrus Leaves and #1577A Bovine Liver were used as quality control materials in the analyses of minerals.

Data were statistically compared by using two-way analysis of variance (ANOVA) *(12)*. Tukey's Studentized range tests were performed when appropriate. Differences between values were considered significant when $p < 0.05$.

RESULTS

The influence of arginine and silicon on Con-A-induced DNA synthesis by splenic T-lymphocytes is shown in Fig. 1. The counts presented represent the DPM of Con-A-stimulated minus Con-A-unstimulated cell cultures. Values are means ± SEM for nine animals. Although arginine significantly decreased Con-A-induced DNA synthesis of splenic T-lymphocytes (p=0.004), the decrease was influenced by dietary silicon. The significant interaction (p=0.05) between silicon and arginine resulted in the decrease being more marked in the silicon-adequate rats. Thus, the DNA synthesis of splenic T-lymphocytes was higher in silicon-adequate than silicon-deficient rats when no supplemental arginine was fed, but in rats fed supplemental arginine, the thymidine incorporation into splenic T-lymphocytes was lower in silicon-adequate than silicon-deficient rats.

Evidence that the animals fed low silicon had an inadequate silicon status was the finding of expected changes in the liver. Silicon deprivation decreased the calcium and phosphorus concentrations of the liver with the
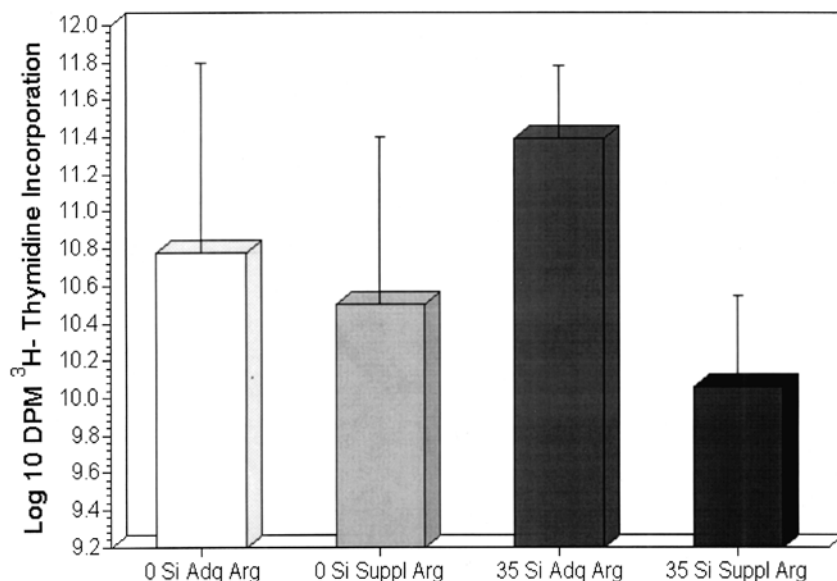
Fig. 1. Influence of arginine and silicon on Con-A-induced DNA synthesis of splenic T-lymphocytes. Cells were stimulated for maximal activity by Con-A at concentrations of 0.1, 0.2, 0.39, 0.78, 1.56, 3.12, or 6.25 μg per 2×10^5 cells in a 200-μL volume. Maximal peak activity shown most frequently occurred in cell cultures stimulated by 0.39 or 0.78 μg Con-A. Count difference represents DPM of Con-A-stimulated minus Con-A-unstimulated cell cultures. Values are means ± SEM for nine animals. The *p*-values for arginine and the interaction between silicon and arginine were 0.004 and 0.05, respectively.

effect most marked in rats not supplemented with arginine (Table 1). In contrast, supplemental arginine increased the liver phosphorus concentrations and increased the calcium concentration in silicon-deficient but not in silicon-supplemented rats. Silicon deprivation also decreased magnesium, manganese, potassium, and zinc concentrations in the liver. Of these four minerals, only the manganese concentration of the liver was significantly increased by supplemental arginine.

The liver weight/body weight ratio of the rats was significantly affected by dietary silicon; it was greater in silicon-adequate than in silicon-deprived rats. Arginine did not affect the liver weight/body weight ratio.

DISCUSSION

The liver mineral concentrations shown in Table 1 indicate that low and adequate intakes of silicon were achieved. Silicon deprivation decreased the concentrations of calcium, phosphorus, potassium, magnesium, and zinc. These are mineral elements that apparently are specifically

Table 1
Effect in Rats of Dietary Arginine and Silicon on Liver Weight/Body Weight Ratio and Liver Mineral Concentrations

Treatment Si μg/g	Arg mg/g	Liver Weight/ Body Weight Ratio	Ca mg/g	P mg/g	K mg/g	Mg mg/g	Zn μg/g	Mn μg/g
0	0	2.75 ± 0.07[a]	118 ± 3	10.81 ± 0.18	10.25 ± 0.14	0.73 ± 0.10	80 ± 2	7.78 ± 0.22
0	5	2.68 ± 0.07	146 ± 3	11.12 ± 0.18	10.24 ± 0.14	0.74 ± 0.10	98 ± 2	8.07 ± 0.22
35	0	2.88 ± 0.07	156 ± 3	11.47 ± 0.18	10.75 ± 0.14	0.79 ± 0.10	103 ± 2	8.01 ± 0.22
35	5	2.81 ± 0.07	153 ± 3	11.91 ± 0.18	11.00 ± 0.14	0.81 ± 0.10	109 ± 2	8.91 ± 0.22

Analyses of Variance - P values

Si		0.05	0.0001	0.0002	.0001	0.0007	0.0001	0.02
Arg		NS	0.0005	0.04	NS	NS	NS	0.008
Si x Arg		NS	0.0001	NS	NS	NS	NS	NS

[a] Means ± pooled SEM (from ANOVA).

required for lymphocyte proliferation *(13,14)*. Arginine supplementation had only a limited effect on these elements; it increased liver phosphorus concentrations and interacted with silicon to affect calcium concentrations. The liver manganese concentration was increased by arginine supplementation and was higher in silicon-adequate than in silicon-deprived rats. Manganese has a key role in glycosylation reactions, which are important in wound healing and bone formation.

The Con-A-induced lymphocyte mitogenesis response to arginine supplementation in the present experiment was somewhat surprising; it was decreased. In humans, arginine supplementation of 17 and 24.8 g/d increased lymphocyte mitogenesis in response to Con-A *(15)*. Arginine supplementation of 25 g/d also enhanced the mean T-lymphocyte response to Con-A of postoperative patients when compared to postoperative patients receiving L-glycine *(16)*. In contrast to these human studies, some animal studies have shown no response to arginine supplementation. Supplemental arginine (3%) fed to aged (24 mo) and young (2 mo) rats did not increase Con-A-stimulated lymphocyte proliferation when the comparison was with rats of similar age fed control (1.12% arginine) diets *(17)*. Other reports are consistent with our findings. Proliferation of splenocytes from chicks fed an arginine-sufficient diet (14.4 g/kg) was greater than that in chicks fed arginine-excess or arginine-deficient diets *(18)*. Inhibition of proliferation in response to mitogen occurred when human lymphocytes were incubated in vitro with pharmacological doses of L-arginine *(19)*. This finding resulted in a suggestion of exercising caution when using L-arginine supplementation to reverse clinical immunosuppression.

The decrease in proliferation of lymphocytes from animals fed excessive arginine in the present study is not unreasonable; it may have been caused by changes in nitric oxide metabolism. Hoffman et al. *(20)* reported findings suggesting that rat lymphocyte proliferation is inhibited by arginine through being affected by cytostatic products of oxidative L-arginine metabolism or through an indirect deleterious effect on mitochondrial respiration.

Perhaps some of the inconsistency in the previously reported responses to arginine supplementation was caused by a variation in silicon status. In the present study, arginine supplementation minimally decreased the proliferation of lymphocytes in response to Con-A when the diet was inadequate in silicon. The most marked decrease occurred in silicon-adequate rats, whose lymphocytes were stimulated much more by Con-A than lymphocytes from silicon-deficient rats when arginine was not excessive. The finding that silicon affected the lymphocyte proliferation when dietary arginine was normal is consistent with findings reported elsewhere. Treatment of patients with a soluble form of silicon in either a drinkable or injectable form increased circulating lymphocytes and immunoglobulins (especially IgG) *(21)*. Human peripheral lymphocytes were stimulated by being cultured in a medium containing 10 mg Si/L; on the other hand, identical conditions inhibited the growth of lymphoblastoid cells *(3)*. Akuginova et al. *(22)* reported that feeding mice a silicon-deficient diet resulted

in splenic lymphocytes with a pronounced decrease in the ability to proliferate in the presence of Con-A and mycobacterial antigens, and even in the absence of stimuli. These workers found that silicon supplementation of the diet restored the immune responses of the splenic lymphocytes.

The mechanisms through which silicon affects lymphocytes have not been clearly defined. There are findings suggesting that silicon upregulates interferon-δ (IFN-δ) production by some lymphocyte phenotypes *(23)*. IFN-δ apparently is an important signal in lymphoycte differentiation. Silicon also influences calcium metabolism as indicated by the liver calcium concentrations given in Table 1. Calcium-dependent nitric oxide synthase activity apparently mediates rat lymphocyte proliferation *(24)*. Also, silica exposure of lymphocytes increases plasma membrane potentials, which increases cytosolic free-calcium concentrations *(25)*. Mitogens such as Con-A trigger an increase in cytosolic calcium, which, apparently, is controlled by membrane potential *(26)*. Blocking of the highly voltage-dependent Ca^{2+}-sensitive K^+ channel blocks the release of IFN-δ from activated T-cells, thus preventing lymphocyte proliferation *(27)*.

Figure 1 shows that the in vitro proliferation was the highest for splenocytes from rats fed the diet with adequate silicon and normal arginine. This suggests that adequate or physiological amounts of both silicon and arginine in the diet are needed to produce optimal antigenic effects and maximum lymphocyte proliferation. On the other hand, when excessive arginine was fed with adequate silicon, lymphocyte proliferation was lowest among the treatment groups. This may have occurred because of a combination of increased oxidative arginine metabolism *(20)* and the stimulation of nitric oxide production from the excessive arginine by silicon. By using the nonselective nitric oxide synthase inhibitor L-NAME, Kang et al. *(28)* showed that macrophages stimulated with silica induced nitric oxide production from arginine and activated the multiprotein complex, NF-κB. NF-κB is an essential transcription factor for the induction of inducible nitric oxide synthase (iNOS) that results in nitric oxide production in macrophages *(29)*. NF-κB apparently also regulates the cationic amino acid transporter and, thus, arginine uptake by cells *(30)*.

Interestingly, the lymphocyte proliferation findings support our suggestion that silicon is needed for optimal wound healing *(31)*. Hoffman et al. *(20)* have suggested that T-lymphocytes at the wound site are crucial for the healing process. Figure 1 shows that maximum proliferation of lymphocytes occurred with adequate silicon in the presence of normal dietary arginine; this indicates that inadequate silicon would hinder wound healing. Also, NF-κB activation apparently is needed for ulcer healing in rats *(32)*; as indicated above, silica is associated with increased NF-κB activation.

In summary, our findings support the hypothesis that silicon is a nutritionally important element. Physiological amounts of silicon affect lymphocyte proliferation and, thus, immune function and wound healing. Furthermore, our findings show that physiological amounts of silicon also influence the proliferation of lymphocytes and, thus, immune reactions in

response to supplemental arginine. The optimal dietary intake of silicon for optimal immune function and the maintenance of health needs to be determined.

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